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CHANGES IN METAL CONCENTRATION IN HEPATIC TISSUE IN SOME LIVER DISEASES
I. ASSESSMENT OF Zn, Cu and Mn CONCENTRATIONS IN NECROTIC MATERIAL[1]

by

M. Kment. M. Cirkt, M. Tichy

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The authors investigated the concentration of Zn, Cu, and Mn in hepatic tissue of subjects with portal cirrhosis, hepatic venostasis and carcinomas without metastases into the liver. Specimens of hepatic tissue were obtained by necropsy. In the group of portal cirrhoses the authors found in hepatic tissue a significantly higher copper concentration compared with all other groups. The Mn concentration was practically the same in all groups investigated.

The attention of many workers is focused on the problem of trace elements in the physiology and pathology of the organism [1,2,3,4,5,6].

I. Internal clinic of the Medical Hygiene Department at Charles University Prague, Head, Professor V. Visek, M.D. Dr. Sc.
Institute of Hygiene and Epidemiology, Prague, director Professor F. Janda M.D., Dr. Sc.
Institute of Work Hygiene and Occupational Diseases, Prague, director Professor B. Svestka, M.D., associate member, Czechoslovak Academy of Sciences.

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Recently, sensitive analytical methods, for example, such methods as atomic absorption spectrophotometry and neutron activation analysis made it possible to determine very low concentrations of the elements in a minimal amount of biological material. Halsted [7], Halsted and Smith [8], found a significant difference in the zinc concentration in plasma in patients with liver diseases and in the control group. Mc Bean et al [9] studied the zinc concentration in human tissues of persons who died from various diseases and in healthy persons who died suddenly in automobile accidents. The authors did not find statistically significant differences between the two groups. The heterogeneity of the first group and the small number of cases, however, did not make it possible to make a statistical analysis of the zinc concentration in different tissues for individual diseases.

In this study we were interested in the copper, zinc and manganese concentration in hepatic tissue and its relation to various diseases of the liver. To have an idea about the concentration of these metals in the hepatic tissue, before we determined these metals in bioptic samples, we began our study with samples from the autopsy material.

Table 1. Classification of the group studied on the basis of the pathological anatomical and histological findings. The columns give the number of cases and the sex of the deceased persons in the individual groups.

Tabulka 1. Rozdělení sledovaného souboru na podkladě patologickoanatomického a histologického nálezu. Ve sloupcích je uveden počet případů a pohlaví zemřelých osob v jednotlivých skupinách

	Počet případů	Pohlaví	
		muži	ženy
Portální cirhóza	10	6	4
Venostatická játra	10	5	5
Karcinomy bez metastáz do jater	6	2	4
Urémie	2	–	2
Krvácení do zažívacího ústrojí	1	1	–
Pneumonie	1	–	1
Chronická cholangoitis	1	–	1
Celkem	31	14	17

Key: a) Number of cases
b) Sex
c) Males
d) Females
e) Portal cirrhosis
f) Hepatic venostatis
g) Carcinomas without metastates into liver
h) Uremias
i) Hemorrhage into digestive system
j) Pneumonias
k) Chronic cholangitis
l) Total

We took 32 liver samples from 31 deceased persons, which were dissected at the Institute of Pathological Anatomy of the Medical Hygiene Department at Charles University in Prague (Head J. Doblas M.D., CSc.) in the period between October 10, 1971 and January 17, 1972. Liver samples weighing 3-10g were taken from the right liver lobe not later than 24 hours after death, from livers with a pathological macroscopic finding, or whenever a disease of the liver was suspected on the basis of clinical symptoms. A part of the sample was processed histologically by the workers in the Institute of Pathological Anatomy. After the moist weight of the tissue was determined, the sample was mineralized by nitric acid using the moist procedure. The mineralized sample was vaporized to the smallest possible volume and the residue was dissolved in deionized water. Next, the sample was vaporized three times in the water bath until it was dry and dissolved in a known volume of deionized water. The manganese, copper and zinc concentrations in the samples were determined without further processing by the atomic absorption spectrophotometry method on a model AA-4 spectrophotometer manufactured by the firm Varian-Techtron. Hollow cathode tubes manufactured by the same firm were used for the manganese, copper and zinc. The conditions for determining the individual metals corresponded to the parameters specified by the manufacturer [10]. The metal concentrations were read off from the

calibration curves. Manganese dichloride [$MnCl_2$, $4H_2O$], electrolytic copper and electrolytic zinc were used as the standard substance.

Table 2. Cu, Zn and Mn concentration (in μm/g moist tissue weight or in mg/total weight of liver) and the Zn:Cu ratio in hepatic tissue in the group of portal cirrhoses, hepatic venostases and carcinomas without metastases into the liver. The hepatic tissue samples were obtained by necropsy. The values presented in the table are mean values and the 95% confidence intervals for the calculated means. The groups were compared using the Duncan test. The thick vertical lines indicate statistical significance ($p < 0.05$).

	a Počet případů	b μg/g vlhké váhy tkáně			Zn:Cu	c mg/váha jater v gramech		
		Cu	Zn	Mn		Cu	Zn	Mn
d Portální cirhóza	10	11,97 ± 1,84	38,85 ± 4,5	1,36 ± 0,19	3,81 ± 0,70	19,27 ± 2,67	66,04 ± 11,76	2,03 ± 0,32
e Venostatická játra	10	6,33 ± 0,81	78,09 ± 9,83	1,49 ± 0,23	12,51 ± 0,88	11,39 ± 1,67	138,63 ± 20,83	2,60 ± 0,54
f Karcinomy bez metastáz do jater	6	5,19 ± 0,92	56,63 ± 5,50	1,49 ± 0,24	11,93 ± 1,55	7,99 ± 1,70	88,61 ± 14,01	2,32 ± 0,41

Key: a) Number of cases
b) μg/g moist tissue weight
c) mg/liver weight in grams.
d) Portal cirrhosis
e) Hepatic venostasis
f) Carcinoma without metastases into liver

The samples were divided on the basis the pathological anatomical and histological findings into 4 groups: portal cirrhoses, hepatic venostases, (venostasis based on ischemic heart disease), carcinomas of lungs, stomach, pancreas, breast), carcinomas of other organs without demonstrable metastases into the liver, and the remaining cases which were not able to classify into any of the previous groups (uremias, pneumonias, hemorrhage into the digestive tract, and chronic cholangoilitis). A summary classification and the number of cases in the individual groups are given in Table 1.

The mean values and the 95% confidence intervals for the means were

determined in the individual groups and the groups were compared using the Duncan test. The statistical department of the Institute of Hygiene and Epidomology carried out the statistical treatment of the material (director Eng. Z. Roth, CSc).

Table 3. Cu, Zn and Mn content (in µg/g moist tissue weight or in mg/total liver weight) and the Zn:Cu ratio in the hepatic tissue in individual cases that were not classified into the groups given in Table 2. The hepatic tissue samples were obtained by necropsy. The values presented in the table are the means and the 95% confidence intervals [Sic] for the calculated means

Tab. 3. Obsah Cu, Zn a Mn (v µg/g vlhké váhy tkáně, resp. v mg/váha celých jater) a poměr Zn:Cu v jaterní tkáni jednotlivých případů, které nebyly zařazeny do skupin uvedených v tabulce 2. Vzorky jaterní tkáně byly získány nekropsií. Hodnoty uvedené v tabulce jsou průměry a 95% intervaly spolehlivosti pro vypočtené průměry

	a µg/g vlhké váhy tkáně			Zn : Cu	b mg/váha jater v gramech		
	Cu	Zn	Mn		Cu	Zn	Mn
c Urémie	3,01	26,44	1,32	8,78	3,70	32,5	1,62
d Urémie	4,08	23,34	0,29	5,72	7,67	43,8	0,54
e Pneumonie	2,65	44,2	0,46	16,67	h játra nevážena		
f Krvácení do zažívacího ústrojí	9,47	79,1	1,93	8,35	i játra nevážena		
g Chronická cholangoitis	3,98	95,18	1,83	23,91	7,16	171,32	3,29

Key: a) µg/g moist tissue weight
b) mg/liver weight in grams.
c) Uremias
d) Uremias
e) Pneumonias
f) Hemorrhage into digestive system
g) Chronic cholangoilitis
h) Liver not weighed
i) Liver not weighed

Results

Table 2 gives the Mn, Cu and Zn content in the hepatic tissue in the individual groups both in µg/g moist tissue and in mg/entire liver. A significant increase in the copper concentration was found in the portal cirrhoses group in comparison with the remaining groups and a significant reduction in the zinc concentration in comparison with the hepatic venostastasis group. No significant differences in the manganese content

were found between the individual groups. The zinc to copper ratio in the portal cirrhoses group was significantly reduced in comparison with the hepatic venastases group and also in comparison with the group of carcinomas without metastases into the liver. The amount of zinc in the entire liver is significantly higher in the hepatic venostases than in the group of carcinomas without metastases into the liver. Table 3 gives the results of measurements of the Cu, Zn and Mn content in the hepatic tissue in the cases which could not be classsified into any of the previous groups.

Discussion

It is clear from our results that in persons suffering from portal cirrhosis of the liver, the zinc concentration in the liver is lower and the copper concentration is higher in comparison with the remaining pathological conditions that were studied. The decrease in the zinc concentration agrees with the results of Vallee [11], while this author did not detect an increase in the copper concentration. According to Gregoriadis and Sourkes [12] the copper accumulates in the liver when the specific transport protein formation responsible for transporting the copper into the bile is reduced. Since it is known that in cirrhoses the formation of non-collagen proteins in the hepatic tissue is repressed [13], it can be assumed that the accumulation of copper in the hepatic tissue may be connected with the elimination of the copper through the bile in patients suffering from cirrhosis. On the other hand, an increase in the copper concentration in the hepatic tissue may contribute to the maturation of collagenic structures in the liver. An oxidative deamination of lysine occurs in the first stage during the formation of the cross-bonds between the collagen molecules, which are the basis for the maturation

process. The enzyme which is responsible for this reaction contains copper [14]. Hence the assumption can be made that the higher copper content in the hepatic tissue stimulates the activity of this enzyme, which indirectly accelerates the maturation of the collagen fibers.

In agreement with Vallee's results [11], the manganese content is identical for all pratical purposes in all the groups studied by us.

Our group included at 76 year old man whose clinical symptoms led us to suspect cirrhosis of the liver, which however was not confirmed macroscopically or histologically. Nevertheless the zinc and copper ratio content and the zinc and copper ratio corresponded to the values found in patients suffering from cirrhosis. Although this patient was not included in the group of patients suffering from cirrhosis, in our opinion, the possibility that this condition could eventually culminate in cirrhosis must not be excluded. In our opinion the changes in the copper and zinc concentrations in the liver may be accompanied by processes resulting in cirrhosis of the liver. We would like to validate this hypothesis in a future experimental study on animals.

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